(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 August 2001 (30.08.2001)

PCT

(10) International Publication Number
WO 01/62261 A1

(51) International Patent Classification[7]: A61K 31/65, A61P 35/00, 11/00, 11/06

(21) International Application Number: PCT/GB01/00814

(22) International Filing Date: 26 February 2001 (26.02.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0004531.0 25 February 2000 (25.02.2000) GB

(71) Applicant *(for all designated States except US)*: **ARAKIS LTD.** [GB/GB]; The Coach House, 88 Long Lane, Willingham, Cambridge CB4 5LD (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **RICHARDS, Andrew, John, McGlashan** [GB/GB]; The Limes, 88 Long Lane, Willingham, Cambridge CB4 5LD (GB). **BANNISTER, Robin, Mark** [GB/GB]; Arakis Ltd., The Coach House, 88 Long Lane, Willingham, Cambridge CB4 5LD (GB). **CHAPLIN, Sharon, Adele** [GB/GB]; Arakis Ltd., The Coach House, 88 Long Lane, Willingham, Cambridge CB4 5LD (GB).

(74) Agent: **GILL JENNINGS & EVERY**; Broadgate House, 7 Eldon Street, London EC2M 7LH (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/62261 A1

(54) Title: METALLOPROTEINASE INHIBITORS FOR THE TREATMENT OF RESPIRATORY DISEASES

(57) Abstract: Use of a compound for the manufacture of a medicament for the treatment of a respiratory disease involving tissue destruction, wherein a compound has an inhibitory activity of greater than 50 % inhibition of MMP1 or MMP2 or MMP8 or MMP9 at less than 100 μM concentration in an enzyme assay and which also downregulates in COPD lung tissue MMP1 or MMP2 or MMP8 or MMP9 to less than 50 % of untreated levels at 100 μM.

METALLOPROTEINASE INHIBITORS FOR THE TREATMENT OF RESPIRATORY DISEASES

Field of the Invention

This invention relates to the treatment of respiratory diseases.

Background of the Invention

Many respiratory diseases have acute components, which include reduction of gaseous exchange due to acute effects involving constriction of the airways. This may be due to infection, bronchoconstriction, excess mucous and other mechanisms. In addition, this is often accompanied by a more serious and irreversible destruction of lung tissue. These effects combined lead to a steady loss of lung function, resulting in lower quality of life and shortened life expectancy. Such diseases include chronic obstructive pulmonary disease (COPD), chronic bronchitis, emphysema, asthma, cystic fibrosis (CF) and lung cancer.

Matrix metalloproteinase enzymes are well known to have a central role in the tissue remodeling process. Inhibitors of these enzymes are under development for a number of therapeutic endpoints including inflammatory diseases (rheumatoid arthritis), oncology and periodontitis. Peptidic inhibitors of MMP enzymes have also been proposed for the treatment of lung diseases including COPD.

The tetracycline antibiotics are a well known class of compounds. They are normally administered as systemic antibiotics by the oral route. Typically, a tablet or capsule containing 50 mg or more of the drug is administered daily over a short period, in order to treat infection. In COPD, the underlying condition (which may have an infectious element) is typically treated using a bronchodilator.

Doxycycline and other tetracyclines are well known as moderate inhibitors of MMP enzymes. Doxycycline is registered, on the basis of this activity, for the treatment of periodontal disease.

US-A-5773430 discloses that certain hydrophobic tetracycline derivatives inhibit serine proteinases and also metalloproteinases. CF is included among the conditions that can be treated. Among the tetracyclines, "doxycline" (sic) is mentioned, but such compounds are considered as unsatisfactory by comparison

with chemically modified tetracyclines and especially 4-de(dimethylamino)-tetracyclines.

US-A-5789395 discloses that tetracycline compounds inhibit NO production.

Summary of the Invention

The present invention is based on studies that provides the first direct evidence concerning the viable efficacy of certain MMP inhibitors, and specifically the utility of doxycycline as a modulator of MMP's and TIMP's, in the treatment of COPD. In particular, and surprisingly, it has been found that doxycycline modulates the levels of MMP enzymes when dosed to diseased lung tissue that has been resected from COPD patients with concurrent lung cancer. Dosing of doxycycline to this tissue reduces the levels of key MMP's implicated in tissue destruction in COPD. Doxycycline demonstrated a significant reduction in levels of MMP enzymes, when compared to control experiments. Decrease in MMP levels has been shown to lead to a direct correlation with the slowing of progression of tissue destruction in analogous connective tissue disorders, for example in arthritis and cancer metastases; see Shalinsky *et al*, Invest. New Drugs (1998-9) 16(4):303-13.

Surprisingly, *in vivo*, in addition to the effect upon MMP-9, it has now been demonstrated that doxycycline promotes significant increases in levels of TIMP-1, the natural inhibitor of MMP-9, thereby potentiating the effect on MMP-9. Clinical data demonstrate for the first time multiple mechanisms of action for doxycycline. An *ex vivo* study shows the modest but useful inhibition of MMP-9 expression/secretion. An *in vivo* study demonstrates that doxycycline also increases the expression/secretion of the natural inhibitor of MMP-9 (TIMP-1). These properties acting in concert allow doxycycline to exert potent MMP inhibitory activity, at doses effective for the treatment of tissue destruction-related diseases.

Further, it is known that oral doxycycline is extensively metabolised in the liver and that, when given by a route of administration that avoids first pass metabolism, lung concentrations of the drug can be up to 10 times the level in plasma; see Bocker *et al*, Arzeneimittelforschung (1981) 31(12):2116-7. In

addition, when given by the oral route, doxycycline is known to be very highly protein-bound, and therefore oral delivery may not be optimal for treating respiratory diseases. Administering doxycycline according to the invention for the treatment of COPD, by the inhaled route, thus maximises the benefit of the newly discovered MMP-modulating activity as well as encouraging high concentrations of drug in diseased tissue and by minimising the exposure of drug in plasma, which may be lost through plasma binding.

These factors work in concert to minimise overall exposure to drug and its unwanted side-effects, which include its antibacterial and systemic side-effects; see BNF (Sept. 2000) 264-5 and Physicians' Desk Reference ed.55 (2001) 1103 (Collagenex) and 2537 (Pfizer). Further such side-effects are due to MMP inhibition and concomitant tissue effects. More particularly, according to the invention, and even when used by the oral route, owing to accumulation in the lung, the drug may be used at doses that are lower than for the treatment of other MMP-mediated diseases.

According to the present invention, doxycycline or another tetracycline antibiotic is used to treat a respiratory disease involving tissue destruction. More generally, based on the information provided herein, the active agent may be any that has an inhibitory activity of greater than 50% inhibition of MMP1 or MMP2 or MMP8 or MMP9 at less than 100 μM concentration in an enzyme assay and which also downregulates in COPD lung tissue MMP1 or MMP2 or MMP8 or MMP9 to less than 50% of untreated levels at 100 μM, and/or an inhibitory activity greater than 50% inhibition of MMP1 or MMP2 or MMP8 or MMP9 at less than 100uM concentration in an enzyme assay which also upregulates TIMP-1 in COPD sputum to more than 200% of untreated levels following repeated dosing at 100 mg once daily.

The criticality of the roles of MMP and TIMP is illustrated by Vignola *et al*, Am. J. Respir. Core Med. (1998) 158(6):1945-50. See Segura-Valdez *et al*, Chest (Mar 2000) 117(3):684-94.

Description of the Invention

A compound suitable for use in the invention may be readily determined by the skilled person, based on the standard assays and other information

provided herein. For example, tetracyclines, including tetracycline antibiotics, are well known. Many such compounds have been disclosed and tested as antibiotics, and are suitable for use in this invention. See also Mitscher, The Chemistry of the Tetracycline Antibiotics, Marcel Dekker, New York (1978), Chapter 6. Examples include doxycycline, tetracycline and minocycline. The preferred active agent for use in this invention is doxycycline, and this drug may be discussed below by way of illustration.

A compound for use in the invention has one or both of the inhibitory profiles given above. In the first such profile, the given concentration is less than 100 μM, preferably less than 50 μM; for doxycycline, the value is about 20 μM. In the second profile, the upregulation is more than 200%, preferably more than 500%; for doxycycline, the value is about 1000%. Preferred compounds for use in the invention meet both these criteria, e.g. having at least one property that is at least as active as doxycycline.

An alternative expression of a compound suitable for use in the invention is that the compound is doxycycline, minocycline or a chemically modified tetracycline which exhibits metalloproteinase inhibitory activity and substantially no antimicrobial activity in a mammalian system. Such tetracyclines are described in US-A-5789395 (the contents of this and other references given herein are incorporated by reference).

For use in this invention, the tetracycline antibiotic is formulated for inhalation or oral administration, and administered to a subject suffering from a respiratory disease involving tissue destruction. The treatment may also address the acute infectious element, but is effective to treat the underlying tissue destruction that is present in some forms of lung disease, including COPD and CF. After a period of treatment, a reduction in infection is noted when the drug is administered at a dose appropriate as an anti-infective. At this dose and doses lower than necessary for use as an antibiotic, the rate of tissue destruction may also be reduced.

Modern methods of delivery of drugs by the inhaled route allow dosing to the lower lung. This may be achieved through control of particle properties (including shape, size and electrostatic forces) using powder or liquid particle

formulation. Suitable particle sizes are up to 1 μm, or up to 5 μm or above, depending on the intended target. Such control can be utilised to deliver doxycycline (by way of example) throughout the lung, and to the lower lung where, through its MMP inhibitory activity, it will slow and potentially reverse the rate of ongoing tissue destruction.

In particular, it has been found that it is possible to formulate tetracyclines in devices suitable for pulmonary delivery, and deliver them topically to the lung. This can be achieved using a range of pulmonary systems and formulation techniques known to those skilled in the art such as, but not limited to, for instance, nebulisers, multi-dose inhalers, dry powder inhalers and pressurised metered multi-dose inhalers. A tetracycline antibiotic such as doxycycline can be readily formulated for inhalation, e.g. with one or more conventional additives such as carriers, excipients, surface active agents etc.

The amount of the active agent to be administered will be determined by the usual factors such as the nature and severity of the disease, the condition of the patient and the potency of the agent itself. These factors can readily be determined by the skilled man. By way of example only, a suitable inhaled daily dose of doxycycline is 1 mg to 50 mg. The amount can be selected such that there is no effective change in airway flora. More particularly, the dosage per inhalation can be less than 20 mg, preferably less than 10 mg, e.g. less than 5 or even less than 2 mg.

Further, the active agent may also be administered by any oral route that provides appropriate drug concentration at the site of lung tissue destruction. Surprisingly, the MMP-lowering effect is seen at doses of below those customarily used to treat infection. Thus, doses below 50 mg (of doxycycline, or an equivalent amount of another suitable drug) can be used by the oral route to treat the tissue destruction seen in COPD. More generally, an oral dosage may be below 200 mg, often below 100 or 50 mg, and may even be below 25, 10, 5 or 1 mg. A suitable formulation for this purpose is a unit dosage such as a tablet or capsule.

The condition to be treated by means of the invention may be, for example, COPD, chronic bronchitis, emphysema, asthma, CF or lung cancer.

These are chronic conditions, and so treatment will generally be for longer than if infection only is treated. Treatment may be for at least 2 or 4 weeks, and generally for longer, e.g. months or even years.

It may be desirable to deliver tetracycline antibiotics to the lung in combination or concomitantly with other agents. These can be bronchodilators (e.g. beta agonists such as salmeterol or terbutaline, or anticholinergics such as ipratropium), anti-inflammatories (e.g. steroids such as budesonide, beclomethasone or fluticasone, leukotriene antagonists and phosphodiesterase 4 inhibitors), anti-trypsin, or other anti-infective agents. In some cases, it may be desirable to formulate the drugs separately within an inhaler device, to achieve different release rates within the lung, dependent on the characteristics of the individual drugs at the site of action.

The studies on which the present invention is based will now be described. The data presented below demonstrate for the first time a multiple mechanism of action for doxycycline. The *in vitro* study shows the modest but useful inhibition of MMP-9 expression/secretion. The *in vivo* study demonstrates that doxycycline also increases the expression/secretion of the natural inhibitor of MMP-9 (TIMP-1). It is these two properties acting in concert that may allow doxycycline to exert surprisingly potent MMP inhibitory activity in the treatment of respiratory diseases.

Lung Tissue Study

This study was done to assess the effects of doxycycline on the release of matrix metalloproteinases by human lung tissue *in vitro*.

Lung tissue from a human who had a greater than 20 pack year history as a smoker, was chopped and incubated overnight in serum-free medium (RPMI 1640) containing penicillin, streptomycin and gentamycin (culture buffer). On the following day, 2-3 fragments (total weight 50 mg) were placed in 0.8 ml of culture buffer and 0.1 ml of doxycycline (to give a final concentration of 10^{-4} – 10^{-9} M) or a buffer control was added.

After one hour's incubation at 37°C, the fragments were treated with either 0.1 ml of either a buffer control (unstimulated fragments) or 1000 U/ml interleukin-1 (final concentration of 100 U/ml IL-1). The fragments were then

incubated for 24 hours and the supernatant recovered; the tissue was weighed and stored at -70°C.

The MMPs and TIMPs released into the supernatant were measured using commercial ELISAs (Amersham); values were expressed as ng of MMP/TIMP per mg of lung tissue.

Human Study

This was an open-label, ascending dose, cross-over study. Suitable subjects with chronic obstructive pulmonary disease (COPD) had their respiratory function assessed at baseline and provided sputum and blood for MMP and doxycycline levels. They then received 50 mg, 100 mg and 200 mg of doxycycline capsules for a period of 3 days in ascending order. At the end of each treatment period a further assessment of respiratory function was performed, and a sputum and blood sample was analysed for MMP, TIMP-1 and doxycycline levels. There was a 4-day wash out period between each treatment period of the study.

The MMPs and TIMPs released were measured using commercial ELISAs as detailed above for the lung tissue study.

MMP-1 Method Details

MMP-1 levels are analysed by Elisa plates (Code No. RPN2610) from Amersham Pharmacia Biotech UK Limited, Amersham Place, Little Chalfont, BUCKS HP7 9NA.

The Elisa is run as per manufacturer's instructions.

SPEC

- This Elisa is specific for total MMP-1; recognising proMMP-1, active MMP-1 and MMP-1/TIMP-1 complex.
- Range: 6.25 – 100 ng/ml.
- Sensitivity: 1.7 ng/ml.
- Suitable for use with cell culture, serum and plasma samples.
- Time: 5.5 hour protocol.
- Store at -15 to -30°C.

MMP-2 Method Details

MMP-2 levels are analysed by Elisa plates (Code No. RPN2617) from Amersham Pharmacia Biotech UK Limited, Amersham Place, Little Chalfont, BUCKS HP7 9NA.

The Elisa is run as per manufacturer's instructions.

SPEC

- This Elisa is specific for proMMP-2; recognising free proMMP-2 and MMP-2 complexed to TIMP-2, but not the active form of MMP-2. No cross-reactivity with MMP-1, 3, 7, 8, 9 and MT1-MMP.
- Range: 1.5-24 ng/ml.
- Sensitivity: 0.37 ng/ml.
- Suitable for use with cell-culture, serum, plasma and tissue samples.
- Time: ~3.5 hour protocol.
- Store at -15 to -30°C.

MMP-9 Method Details

MMP-9 levels are analysed by Elisa plates (Code No. RPN2614) from Amersham Pharmacia Biotech UK Limited, Amersham Place, Little Chalfont, BUCKS HP7 9NA.

The Elisa is run as per manufacturer's instructions.

SPEC

- This Elisa is specific for free pro-MMP-9 and pro-MMP-9 complexed to TIMP-1. No cross-reactivity with pro-MMP-1, 2, 3, TIMP-1 and 2.
- Range: 1-32 ng/ml.
- Sensitivity: 0.6 ng/ml.
- Suitable for use with cell-culture supernatant and plasma samples.
- Time: ~4 hour protocol.
- Store at -15 to -30°C.

TIMP-1 Method Details

TIMP-1 levels are analysed by Elisa plates (Code No. RPN2611) from Amersham Pharmacia Biotech UK Limited, Amersham Place, Little Chalfont, BUCKS HP7 9NA.

The Elisa is run as per manufacturer's instructions.

SPEC

- This Elisa is specific for total TIMP-1, free TIMP-1 and TIMP-1 complexed to MMPs.
- Range: 3.13 – 50 ng/ml.
- Sensitivity: 1.25 ng/ml.
- Suitable for use with cell-culture supernatant, serum and plasma samples.
- Time: 4 – 5 hour protocol.
- Store at -15 to -30°C.

Results

Table I – MMP-1 Lung Tissue Study

% of control		% of IL-1	
+10-4 dox	24.05	+10-4 dox	14.75
+10-6 dox	84.10	+10-6 dox	61.47
+10-8 dox	76.50	+10-8 dox	51.87

Table II – MMP-2 Lung Tissue Study

% of control		% of IL-1	
+10-4 dox	34.34	+10-4 dox	49.23
+10-6 dox	219.33	+10-6 dox	86.86
+10-8 dox	101.47	+10-8 dox	95.75

Table III – MMP-9 Lung Tissue Study

% of control		% of IL-1	
+10-4 dox	39.36	+10-4 dox	51.04
+10-6 dox	118.84	+10-6 dox	99.29
+10-8 dox	113.57	+10-8 dox	127.04

Table IV –TIMP-1 Lung Tissue Study

% of control		% of IL-1	
+10-4 dox	44.49	+10-4 dox	75.47
+10-6 dox	182.40	+10-6 dox	508.92
+10-8 dox	172.02	+10-8 dox	461.92

Table V – MMP-9 & TIMP-1 Human Study

Day of Study	% of control	
	MMP-9	TIMP-1
4	39.43	116.24
8	12.85	1101.03
11	29.17	1528.49
15	22.41	974.24
18	29.03	1173.36
Mean	26.58	978.67

The lung tissue study shows that lung tissue treated with 10^{-4} doxycycline shows a dramatic decrease in MMP-1, 2 & 9.

The human patient study shows a 73% reduction in MMP-9 activity and a 10-fold increase in TIMP-1.

CLAIMS

1. Use of a compound for the manufacture of a medicament for the treatment of a respiratory disease involving tissue destruction, wherein the compound has an inhibitory activity of greater than 50% inhibition of MMP1 or MMP2 or MMP8 or MMP9 at less than 100 μM concentration in an enzyme assay and which also downregulates in COPD lung tissue MMP1 or MMP2 or MMP8 or MMP9 to less than 50% of untreated levels at 100 μM.

2. Use of a compound for the manufacture of a medicament for the treatment of a respiratory disease involving tissue destruction, wherein the compound has an inhibitory activity of greater than 50% inhibition of MMP1 or MMP2 or MMP8 or MMP9 at less than 100uM concentration in an enzyme assay which also upregulates TIMP-1 in COPD sputum to more than 200% of untreated levels following repeated dosing at 100 mg once daily.

3. Use of a compound for the manufacture of a medicament for the treatment of a respiratory disease involving tissue destruction, wherein the compound has the activity given in claim 1 and the activity given in claim 2.

4. Use of a tetracycline antibiotic for the manufacture of a medicament for the treatment of a respiratory disease involving tissue destruction.

5. Use of a tetracycline compound for the manufacture of a medicament for the treatment of a respiratory disease involving tissue destruction, wherein the compound is doxycycline, minocycline or a chemically modified tetracycline which exhibits metalloproteinase inhibitory activity and substantially no antimicrobial activity in a mammalian system.

6. Use according to any preceding claim, wherein the compound is doxycycline.

7. Use according to any preceding claim, wherein the disease is a chronic condition.

8. Use according to any preceding claim, wherein the disease is COPD, chronic bronchitis, emphysema, asthma, cystic fibrosis or lung cancer associated with COPD.

9. Use according to any preceding claim, wherein the disease is essentially free of infection requiring an antibiotic.

10. Use according to any preceding claim, wherein the treatment does not affect airway flora.

11. Use according to any preceding claim, wherein the compound is used in combination with or concomitantly with an additional agent selected from steroids, PDE 4 inhibitors, sympathomimetic agents, anti-cholinergics, bronchodilators, theophylline, elastase inhibitors, leukotriene antagonists, and anti-inflammatories.

12. Use according to any preceding claim, wherein the compound is used in combination with another anti-infective agent.

13. Use according to any preceding claim, wherein the medicament is in the form of a unit dosage that contains the compound in an amount that is less than 20 mg of doxycycline or an equipotent equivalent of another compound.

14. Use according to claim 13, wherein the amount is less than 10 mg of doxycycline or equipotent equivalent.

15. Use according to any preceding claim, wherein the medicament is adapted for administration by inhalation.

16. Use according to any of claims 1 to 14, wherein the medicament is adapted for oral administration.

17. A formulation of a compound as defined in any of claims 1 to 6, suitable for administration via an inhalation device.

18. A formulation according to claim 17, in the form of a medicament as defined in claim 13 or claim 14.

19. An inhaler device comprising a formulation according to claim 17 or claim 18.

20. A formulation of a compound as defined in any of claims 1 to 6, suitable for administration via the oral route, in the form of a unit dosage as defined in claim 13 or claim 14.

INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/GB 01/00814

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/65 A61P35/00 A61P11/00 A61P11/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, EMBASE, MEDLINE, CANCERLIT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 789 395 A (ABRAMSON STEVEN B ET AL) 4 August 1998 (1998-08-04) cited in the application	1-11
Y	* see claims 1,3,4 and 8, col.7 line 58 to col.8 line 3, col.4 lines 4-15 *	1-20

X	WO 98 52575 A (CHADA KIRAN K ;UNIV COLUMBIA (US); ARMIENTO JEANINE M D (US)) 26 November 1998 (1998-11-26)	1-5, 7-10, 15-18
Y	* see cl. 1,4-6,10, pages 12, 23 and 25 *	1-20

X	WO 00 09492 A (LETAVIC MICHAEL ANTHONY ;MCCLURE KIM FRANCIS (US); NOE MARK CARL () 24 February 2000 (2000-02-24) * see cl.1, cl.52, page 2 line 24 and pages 24-25 *	1-3,7,8, 11,16

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

°A° document defining the general state of the art which is not considered to be of particular relevance

°E° earlier document but published on or after the international filing date

°L° document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

°O° document referring to an oral disclosure, use, exhibition or other means

°P° document published prior to the international filing date but later than the priority date claimed

°T° later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

°X° document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

°Y° document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

°&° document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 May 2001	05/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040. Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Merckling, V

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/GB 01/00814

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 773 430 A (RAMAMURTHY NUNGAVARAM S ET AL) 30 June 1998 (1998-06-30) cited in the application * see cl.1-2, cl.14, col.6 nd col.1 lines 29-31 *	1-5, 7-10,13, 14,16
X	US 5 856 315 A (BACKER JOSEPH M ET AL) 5 January 1999 (1999-01-05) * see col.1, col.4 lines 33-37, col.5 lines 13-14, col.6 lines 4-10 *	1-5, 7-11,16
X	WO 99 16441 A (GRAMS FRANK ;BRUNNER ALFRED (DE); KRELL HANS WILLI (DE); ROCHE DIA) 8 April 1999 (1999-04-08) * see pages 1 and 18, page 15 lines 26-27 and cl.9 *	1-3, 7-10,16

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ational Application No
PCT/GB 01/00814

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5789395 A	04-08-1998	AU 718234 B	13-04-2000
		AU 4080897 A	19-03-1998
		EP 0966525 A	29-12-1999
		US 5919775 A	06-07-1999
		WO 9808480 A	05-03-1998
WO 9852575 A	26-11-1998	AU 7688998 A	11-12-1998
WO 0009492 A	24-02-2000	AU 4925099 A	06-03-2000
US 5773430 A	30-06-1998	AU 6669898 A	29-09-1998
		EP 0975349 A	02-02-2000
		WO 9840079 A	17-09-1998
US 5856315 A	05-01-1999	US 5843925 A	01-12-1998
WO 9916441 A	08-04-1999	AU 9747098 A	23-04-1999